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ORIGINAL ARTICLE

Prevalence, species distribution and antimicrobial resistance of enterococci isolated from US dairy cattle

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Abstract

Aims: To estimate prevalence and antimicrobial susceptibility of enterococci in faeces collected in 2007 from U.S. dairy cattle.
Methods and Results: A total of 718 faecal samples from 122 dairy cattle operations from 17 US States were collected and cultured for the presence of enterococci. One hundred and eighteen of the 122 operations (96·7%) had at least one dairy cow positive for enterococci and 88·7% (637 of 718) of the faecal samples were positive. At least ten different enterococcal species were found on the dairy operations and 90·7% (107 of 118) of the operations were positive for *Enterococcus hirae* followed by *E. faecalis* (40·7%; 48 of 118) and *E. faecium* (39%; 46 of 118). The highest percentage of resistant isolates were to lincomycin (92·3%; 587 of 636), flavomycin (71·9%; 457 of 636) and tetracycline (24·5%; 156 of 636). Multi-drug resistance (MDR) (resistance ≥ 2 antimicrobials) was observed to as many as seven antimicrobials regardless of class.
Conclusion: In contrast to previous studies, faecal shedding of enterococci in dairy cattle occurred in almost 90% of cows sampled and represented a variety of enterococcal species.
Significance and Impact of Study: Although this study demonstrated a high prevalence of antimicrobial-resistant enterococci from dairy cattle faeces in the United States, the contribution of dairy cattle as a source of antimicrobial-resistant enterococci that can be transmitted to humans remains unclear.

Introduction

Enterococci have been found in a number of environments including the intestinal tract of mammals, soil, water, plants and insects (Witte *et al.* 1999; Muller *et al.* 2001; Aarestrup *et al.* 2002). Although enterococci are ubiquitous in nature and normal commensals in the digestive tract, they are also of medical importance. Enterococci are a leading cause of nosocomial infections in humans and have been indicated in sporadic infections in animals including food animals (Martone 1998; Cetinkaya *et al.* 2000; Kuhn *et al.* 2000). In cattle, enterococci have been associated with diarrhoea in calves and bovine mastitis in dairy cattle (Madsen *et al.* 1974; Rogers *et al.* 1992). Of cases of mastitis where a causative agent has been identified, 2–20% of those were caused by enterococci (Poutrel and Ryniewicz 1984; Aarestrup *et al.* 1995; Sobiraj *et al.* 1997). Enterococci implicated in mastitis are considered environmental pathogens as they are transmitted between the environment and the animal rather than from animal to animal (Rossitto *et al.* 2002).

In addition to their importance in disease, enterococci are also important because of their ability to harbour antimicrobial resistance genes (Murray 1990; Klare *et al.* 2001). The possibility of transfer of antimicrobial-resistant bacteria (pathogens or commensal organisms) from animals to humans has caused increased interest in antimicrobials that are used in both human and veterinary medicine. On dairy farms, mastitis is one of the leading causes of antimicrobial use (Mitchell *et al.* 1998; USDA 2005, 2008a). In the United States, commercial milk is treated by high-temperature, short-time pasteurization

which has resulted in less than 1% of human illness traced to tainted milk (Stabel 2003). Transmission of resistant bacteria to humans may still occur via raw milk products contaminated with resistant bacteria from sub-clinical or latent mastitis infections (Tenhagen *et al.* 2006).

Studies on the prevalence of enterococci in dairy cattle have been reported, but have mainly focused on mastitis and contamination of raw milk products; few studies have addressed the prevalence of enterococci in the faeces of dairy cattle (Aarestrup *et al.* 1995; Rossitto *et al.* 2002; Makovec and Ruegg 2003). A study on the occurrence of enterococci in the faeces of dairy cattle determined that the presence and diversity of species of enterococci in the faeces of adult dairy cows was rare (Devriese *et al.* 1992). In that study, only three enterococcal species, *Enterococcus hirae*, *E. faecalis* and *E. casseliflavus* were isolated in very low numbers. Few other studies have included data on antimicrobial resistance in enterococci isolated from dairy cattle (Gianneechini *et al.* 2002; Pitkala *et al.* 2004; Hershberger *et al.* 2005; Ebrahimi *et al.* 2008). In a more recent study, *E. faecium* and *E. durans* were isolated and tested for susceptibility to a panel of 16 antimicrobials (Edrington *et al.* 2009). *E. faecium* isolates were resistant to nine antimicrobials, while *E. durans* isolates were resistant to five antimicrobials tested in the study. Although these studies examined prevalence and antimicrobial susceptibility separately, none of the studies have included both prevalence and antimicrobial resistance data for enterococci from dairy cattle. In this study, prevalence and antimicrobial resistance of enterococci in faecal samples from cows on US dairy operations participating in the National Animal Health Monitoring System (NAHMS) Dairy 2007 study were examined.

Materials and methods

Sample collection, isolation, and identification of enterococci

The NAHMS Dairy 2007 study represented 79·5% of U.S. dairy operations and 82·5% of US dairy cows and was conducted in 17 states including California, Idaho, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New Mexico, New York, Ohio, Pennsylvania, Texas, Vermont, Virginia, Washington and Wisconsin (USDA 2008b). Approximately 30–35 healthy cows were sampled on each of 122 dairy operations from the end of February to August 2007. Of the 30–35 cows sampled, up to six faecal samples from each operation were tested for the presence of enterococci. For isolation, faecal samples were diluted 1:9 (w/v) in sterile phosphate-buffered saline (PBS, 0·1 mol l^{-1}, pH 7·2). Aliquots (100 μl^{-1}) were inoculated into 24-well tissue culture plates (Becton Dickinson Labware, Franklin Lakes, NJ, USA) containing 1 ml of Enterococcosel broth (Becton Dickinson, Sparks, MD, USA) per well. The enrichment broth was incubated for 18–24 h at 37°C. Positive cultures were transferred to Enterococcosel Agar (Becton Dickinson) for isolation of enterococci. Plates were incubated overnight at 37°C. One presumptive positive colony was passed to blood agar, and the resulting clones were identified to enterococcal genus and species using multiplex PCR as previously described (Jackson *et al.* 2004).

Antimicrobial susceptibility

Minimum inhibitory concentrations (MIC, $\mu g\ ml^{-1}$) for enterococci were determined by broth microdilution using the Sensititre™ semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH, USA) and the Sensititre™ Gram-Positive Custom Plate CMV2AGPF according to the manufacturer's directions. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines when defined [Clinical and Laboratory Standards Institute (CLSI) (2006, 2007)]. No CLSI interpretive criteria have been defined for flavomycin, kanamycin, lincomycin and tylosin, and only susceptible breakpoints have been established for daptomycin (≤4 $\mu g\ ml^{-1}$) and tigecycline (≤0·25 $\mu g\ ml^{-1}$). Breakpoints for daptomycin, flavomycin, kanamycin, lincomycin, tigecycline and tylosin were those defined by the National Antimicrobial Resistance Monitoring System (NARMS) (http://www.ars.usda.gov/Main/docs.htm?docid=6750&page=3). The panel of 17 antimicrobials and breakpoints for classification as resistant used by the NARMS program were as follows: chloramphenicol (≥32 $\mu g\ ml^{-1}$), ciprofloxacin (≥4 $\mu g\ ml^{-1}$), daptomycin (≥8 $\mu g\ ml^{-1}$), erythromycin (≥8 $\mu g\ ml^{-1}$), flavomycin (≥32 $\mu g\ ml^{-1}$), gentamicin (≥500 $\mu g\ ml^{-1}$), kanamycin (≥500 $\mu g\ ml^{-1}$), lincomycin (≥4 $\mu g\ ml^{-1}$), linezolid (≥8 $\mu g\ ml^{-1}$), nitrofurantoin (≥128 $\mu g\ ml^{-1}$), penicillin (≥16 $\mu g\ ml^{-1}$), streptomycin (≥1000 $\mu g\ ml^{-1}$), Synercid (quinupristin/dalfopristin) (≥4 $\mu g\ ml^{-1}$), tetracycline (≥16 $\mu g\ ml^{-1}$), tigecycline (≥0·5 $\mu g\ ml^{-1}$), tylosin (≥32 $\mu g\ ml^{-1}$) and vancomycin (≥32 $\mu g\ ml^{-1}$). *Enterococcus faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were quality controls for determination of MIC.

Results

Prevalence and identification of enterococci

One hundred and eighteen of the 122 operations (96·7%) had at least one dairy cow positive for enterococci. Ten different enterococcal species were identified from the 118

Table 1 Prevalence of enterococci on dairy farm operations

	Isolates (n = 636)		Operations (n = 118)	
Species	No.	Pct.	No.	Pct.
E. hirae	313	49·2	107	90·7
E. faecalis	90	14·2	48	40·7
E. faecium	85	13·4	46	39·0
E. casseliflavus	66	10·4	42	35·6
E. species	23	3·6	19	16·1
E. durans	22	3·5	17	14·4
E. mundtii	20	3·1	16	13·6
E. gallinarum	13	2·0	11	9·3
E. avium	3	0·5	1	0·8
E. flavescens	1	0·2	1	0·8
Total	636	100	–	–

culture-positive dairy operations (Table 1). The majority of positive operations (90·7%; 107 of 118) were positive for *Enterococcus hirae* followed by *E. faecalis* (40·7%; 48 of 118) and *E. faecium* (39%; 46 of 118) (Table 1). Of the 718 faecal samples from the 122 dairy operations tested for the presence of enterococci, 88·7% (637 of 718) were positive for the bacteria. One isolate from the study could not be recovered after freezing and was thus excluded from the results (n = 636).

Results of prevalence of enterococcal species from samples roughly resembled the results obtained from prevalence by operation. The majority of samples were positive for one of three enterococcal species (*E. hirae*, *E. faecalis*, and *E. faecium*). The most prevalent enterococcal species detected was *E. hirae*, which represented almost half (49·2%; 313 of 636) of the isolates (Table 1). *E. faecalis* and *E. faecium* were isolated from 14·2% (90 of 636) and 13·4% (85 of 636) of positive faecal samples, respectively.

Antimicrobial resistance

Of the ten enterococcal species detected, most isolates from all ten species exhibited resistance to lincomycin (Table 2). Resistance to flavomycin and tetracycline was also observed in diverse species as isolates of eight different species were resistant to each of these antimicrobials. In addition, overall resistance was to those three antimicrobials as the majority of isolates were resistant to lincomycin (92·3%; 587 of 636) followed by flavomycin (71·9%; 457 of 636) and tetracycline (24·5%; 156 of 636) (Table 2). In contrast, very few isolates (≤10 per antimicrobial) were resistant to penicillin, streptomycin, kanamycin, erythromycin, quinupristin/dalfopristin, and nitrofurantoin, and none of the isolates were resistant to chloramphenicol, gentamicin, linezolid, tigecycline or vancomycin.

Table 2 Antimicrobial resistance of enterococci isolated from dairy cows

		No. resistant (%)†									
Antimicrobial*/ No. resistant	Breakpoint (μg/ml)	*E. hirae* (n = 313)	*E. faecalis* (n = 90)	*E. faecium* (n = 85)	*E. casseliflavus* (n = 66)	*E. species* (n = 23)	*E. durans* (n = 22)	*E. mundtii* (n = 20)	*E. gallinarum* (n = 13)	*E. avium* (n = 3)	*E. flavescens* (n = 1)
Ciprofloxacin (n = 15)	≥4	0 (0)	0 (0)	13 (15·3)	1 (1·5)	1 (4·3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Daptomycin (n = 17)	≥8	16 (5·1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)	0 (0)
Erythromycin (n = 8)	≥8	5 (1·6)	1 (1·1)	1 (1·2)	0 (0)	1 (4·3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Flavomycin (n = 457)	≥32	258 (82·4)	0 (0)	75 (88·2)	63 (95·5)	19 (82·6)	8 (36·4)	20 (100)	13 (100)	0 (0)	1 (100)
Kanamycin (n = 3)	≥500	1 (0·3)	0 (0)	2 (2·4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lincomycin (n = 587)	≥4	293 (93·6)	88 (97·8)	69 (81·2)	61 (92·4)	20 (87)	22 (100)	19 (95)	12 (92·3)	2 (66·7)	1 (100)
Nitrofurantoin (n = 10)	≥128	2 (0·6)	0 (0)	5 (5·9)	0 (0)	3 (13)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Penicillin (n = 1)	≥16	0 (0)	0 (0)	1 (1·2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Streptomycin (n = 2)	≥1000	1 (0·3)	0 (0)	1 (1·2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Synercid‡ (n = 5)	≥4	3 (1)	NA	0 (0)	0 (0)	1 (4·3)	1 (4·5)	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline (n = 156)	≥16	119 (38)	11 (12·2)	7 (8·2)	3 (4·5)	4 (17·4)	8 (36·4)	2 (10)	2 (15·4)	0 (0)	0 (0)
Tylosin (n = 13)	≥32	4 (1·3)	1 (1·1)	0 (0)	5 (7·6)	2 (8·7)	0 (0)	0 (0)	1 (7·7)	0 (0)	0 (0)

*No isolates were resistant to chloramphenicol, gentamicin, linezolid, tigecycline or vancomycin.
†Per cent resistant was determined by dividing the number of resistant isolates by the total number of isolates per species.
‡*E. faecalis* are intrinsically resistant to Synercid (quinupristin/dalfopristin).

Table 3 Multiple antimicrobial resistance among enterococci isolated from dairy cattle

Species	No. antimicrobials	No. resistant (%)							
		0	1	2	3	4	5	6	7
E. hirae (*n* = 313)		2 (0·6)	38 (12·1)	173 (55·3)	89 (28·4)	7 (2·2)	2 (0·6)	1 (0·3)	1 (0·3)
E. faecalis (*n* = 90)		1 (1·1)	79 (87·8)	9 (10)	0 (0)	1 (1·1)	0 (0)	0 (0)	0 (0)
E. faecium (*n* = 85)		0 (0)	12 (14·1)	61 (71·8)	10 (11·8)	1 (1·2)	0 (0)	1 (1·2)	0 (0)
E. casseliflavus (*n* = 66)		1 (1·5)	5 (7·6)	52 (78·8)	8 (12·1)	0 (0)	0 (0)	0 (0)	0 (0)
E. species (*n* = 23)		1 (4·3)	2 (8·7)	14 (60·9)	3 (13)	3 (13·0)	0 (0)	0 (0)	0 (0)
E. durans (*n* = 22)		0 (0)	8 (36·4)	11 (50)	3 (13·6)	0 (0)	0 (0)	0 (0)	0 (0)
E. mundtii (*n* = 20)		0 (0)	1 (5)	16 (80)	3 (15)	0 (0)	0 (0)	0 (0)	0 (0)
E. gallinarum (*n* = 13)		0 (0)	1 (7·7)	9 (69·2)	3 (23·1)	0 (0)	0 (0)	0 (0)	0 (0)
E. avium (*n* = 3)		1 (33·3)	2 (66·7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
E. flavescens (*n* = 1)		0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total (*n* = 636)		6	148	346	119	12	2	2	1

vancomycin. Of the 17 antimicrobials tested, *E. hirae* isolates as a group were resistant to ten different antimicrobials including daptomycin, erythromycin, flavomycin, kanamycin, lincomycin, nitrofurantoin, streptomycin, quinupristin/dalfopristin, tetracycline and tylosin (Table 2). Interestingly, some *E. hirae* isolates were also resistant to the newer antimicrobial, daptomycin and accounted for 94·1% of the resistance to daptomycin (Table 2). One *E. mundtii* isolate was the only other enterococcal species to exhibit resistance to daptomycin.

Of the 636 isolates tested for antimicrobial susceptibility, six isolates (0·9%) were susceptible to all 17 antimicrobials against which they were tested (Table 3). The majority of *E. faecalis* (87·8%; 79 of 90), *E. avium* (66·7%; 2 of 3) and *E. durans* (36·4; 8 of 22) were resistant to only one antimicrobial, while the majority of isolates for the remaining species were resistant to two antimicrobials (Table 3). Multi-drug resistance (MDR) up to seven antimicrobials was observed with fewer species exhibiting MDR as the number of antimicrobials increased. Isolates of *E. hirae*, *E. faecalis*, *E. faecium* and *E.* species-undetermined were resistant to four antimicrobials, and a single isolate (*E. hirae*) was resistant to seven antimicrobials. *E. hirae* was the only species exhibiting both pan-susceptibility and MDR to seven antimicrobials (Table 3).

Twenty-five different MDR patterns were detected (Table 4). Most of the patterns were composed of either two or three antimicrobials with only one pattern (one isolate) containing seven antimicrobials. This *E. hirae* isolate was resistant to erythromycin, kanamycin, lincomycin, streptomycin, tetracycline, tylosin and quinupristin/dalfopristin (Table 4). The majority of patterns also contained only one or two different enterococcal species, but one pattern, FlaLin, followed by FlaLinTet and LinTet contained the highest number of different enterococcal species (Table 3). Pattern FlaLin contained isolates of eight enterococcal species and FlaLinTet and LinTet each contained six enterococcal species. Four species, *E. casseliflavus*, *E. durans*, *E. faecium* and *E. hirae*, were common between the three patterns. The three antimicrobials (flavomycin, lincomycin and tetracycline) comprising these two patterns reflected the diversity in enterococcal species as many different enterococcal species were resistant to these drugs.

Discussion

In previous studies, either the prevalence or antimicrobial resistance of enterococci from dairy cattle have been examined, but in separate reports (Devriese *et al.* 1992; Hershberger *et al.* 2005; Tenhagen *et al.* 2006). In addition, many of those studies have reported on enterococci from bovine mastitis or milk samples and not enterococci from dairy cattle faecal samples. In this study, both the prevalence and antimicrobial resistance of enterococci from the NAHMS Dairy 2007 study of US dairy operations were examined. The data gathered from this study allowed determination of enterococcal species present in the faeces of dairy cattle across a wide geographical area and under a number of different management systems as well as their antimicrobial resistance patterns. This information will be helpful in future risk assessments of antimicrobial use practices and public health. Because commensal bacteria such as enterococci have natural gene transfer mechanisms and can harbour multiple resistances, it is important to characterize the strains that are isolated from food animals.

In previous studies on the prevalence of enterococci from dairy cattle faecal samples, few enterococcal species were isolated (Devriese *et al.* 1992; Rossitto *et al.* 2002; Kagkli *et al.* 2007; Edrington *et al.* 2009). In those studies, only five enterococcal species, *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium* and *E. hirae*, were isolated. In the

Table 4 Multi-drug resistance patterns in enterococci isolated from dairy cattle

Pattern*,†	No. resistances	Species	Total no.
CipFla	2	*E. faecium*	8
CipFlaLin	3	*E. casseliflavus*	1
		E. faecium	3
CipFlaNit	3	*E. faecium*	1
CipFlaTet	3	*E. faecium*	1
		E. species	1
DapFla	2	*E. hirae*	1
DapFlaLin	3	*E. hirae*	9
		E. mundtii	1
DapFlaLinTet	4	*E. hirae*	6
EryFlaKanLin	4	*E. faecium*	1
EryFlaLinTetTyl	5	*E. hirae*	1
EryFlaLinTetTylSyn	6	*E. hirae*	1
EryFlaLinTyl	4	*E.* species	1
EryKanLinStrTetTylSyn	7	*E. hirae*	1
EryLinTetTyl	4	*E. faecalis*	1
EryLinTetTylSyn	5	*E. hirae*	1
FlaKanLinPenStrTet	6	*E. faecium*	1
FlaLin‡	2	*E. casseliflavus*	51
		E. durans	5
		E. faecium	49
		E. flavescens	1
		E. gallinarum	9
		E. hirae	144
		E. mundtii	16
		E. species	13
FlaLinNit	3	*E. faecium*	3
		E. hirae	1
		E. species	1
FlaLinNitSyn	4	*E.* species	1
FlaLinNitTet	4	*E. hirae*	1
		E. species	1
FlaLinTet	3	*E. casseliflavus*	2
		E. durans	3
		E. faecium	2
		E. gallinarum	2
		E. hirae	79
		E. mundtii	2
FlaLinTyl	3	*E. casseliflavus*	5
		E. gallinarum	1
		E. species	1
FlaTet	2	*E. faecium*	2
		E. hirae	2
LinNit	2	*E. faecium*	1
LinSyn	2	*E. durans*	1
LinTet	2	*E. casseliflavus*	1
		E. durans	5
		E. faecalis	9
		E. faecium	1
		E. hirae	26
		E. species	1

*Cip, ciprofloxacin; Dap, daptomycin; Ery, erythromycin, Fla, flavomycin; Kan, kanamycin; Lin, lincomycin; Nit, nitrofurantoin; Pen, penicillin; Str, streptomycin; Syn, Synercid (quinupristin/dalfopristin); Tet, tetracycline; Tyl, tylosin.

†Synercid was omitted from patterns, where *E. faecalis* was the sole species exhibiting resistance as *E. faecalis* are intrinsically resistant to Synercid.

‡Pattern with highest number of different enterococcal species.

present study, five additional enterococcal species, *E. avium*, *E. flavescens*, *E. gallinarum*, *E. mundtii* and *E.* species (unidentified), in addition to those above were isolated. Furthermore, few of the dairy cattle sampled in those studies were positive for enterococci. This differs dramatically from the results of this study where 96·7% of the dairy operations and 88·7% of the faecal samples were positive for enterococci. These differences could be because of the higher number of animals sampled in this study as well as the geographical locations and number of dairy operations tested. Differences in methodology including sampling, bacterial isolation and species identification could also account for the differences (Hudson *et al.* 2003; Jackson *et al.* 2005).

For the antimicrobials tested, the highest levels of resistance were to lincomycin and flavomycin. For treatment of mastitis in dairy cattle in some European countries, the combination of lincomycin and neomycin is used and found to be effective against *Escherichia coli* and *Staphylococcus aureus* (De Oliveira *et al.* 2000). In the NAHMS Dairy 2007 study, a lincosamide was the primary antibiotic used to treat mastitis on 15·8 per cent of operations (19·4% of cows with mastitis) (USDA 2008b). With the exception of *E. durans*, most enterococci have been reported to be intrinsically resistant to lincomycin (Toala *et al.* 1969; Karchmer *et al.* 1975). In this study, all *E. durans* were resistant to lincomycin, but lower levels of resistance (4·5%) have been reported for *E. durans* from dairy faecal samples in a previous study (Edrington *et al.* 2009). Lower levels of resistance to lincomycin (62%) have also been reported from *E.* species isolated from dairy milk samples (Makovec and Ruegg 2003). For flavomycin, previous studies have reported intrinsic resistance in enterococci (Butaye *et al.* 2003) and almost 72% of enterococci were resistant to flavomycin in that study. Other gram-positive bacteria such as *S. aureus*, classified as a contagious mastitis pathogen, are usually susceptible to flavomycin. Although flavomycin is not used in dairy cows, it can be fed to heifers as a growth-promotant (Rossitto *et al.* 2002; Butaye *et al.* 2003).

One of the most widely used antimicrobial combinations for dry cow treatment and prevention of mastitis is penicillin and novobiocin (De Oliveira *et al.* 2000). Although novobiocin was not tested on the panel of antimicrobials, only one isolate from this study (0·16%) was resistant to penicillin suggesting that the combination therapy would be effective against these isolates. In the NAHMS 2007 study, penicillin G (procaine)/dihydrostreptomycin (along with cephapirin) was also one the most commonly used dry cow antibiotics (31·0 and 36·9% of cows, respectively). Levels of resistance to penicillin in other reports were higher than in the present study (Rossitto *et al.* 2002; Makovec and Ruegg 2003;

Edrington *et al.* 2009). In those studies, 2·5% of enterococci isolated from dairy cattle with mastitis, 9% of *E. faecium* from dairy faecal samples and almost 46% of *E.* species from dairy cow milk samples were resistant to penicillin. Among other antimicrobials tested in the present study, no resistance was found to chloramphenicol, gentamicin, linezolid, tigecycline or vancomycin. Gentamicin resistance in *E. faecalis* and *E. faecium* from dairy cattle has been previously reported, and gentamicin resistance was higher on dairy farms that reported using gentamicin (Hershberger *et al.* 2005). Although only 17 enterococcal isolates were resistant to the newer antimicrobial daptomycin, surprisingly, 16 of those resistant isolates were *E. hirae*. Daptomycin is a lipopeptide antimicrobial approved for treatment of complicated skin and skin-structure infections caused by *Staphylococcus aureus* (methicillin susceptible and resistant), *Streptococcus spp.* (*S. pyogenes, S. agalactiae* and *S. dysgalactiae* subsp. equisimilis) and *Enterococcus faecalis* (vancomycin-susceptible) (Shoemaker *et al.* 2006). Although resistance has been reported in isolates of *E. faecium* and *E. faecalis*, resistance appeared to emerge during drug treatment and resistance of *E. hirae* to daptomycin has not been reported to date (Shoemaker *et al.* 2006).

Multi-drug resistance (MDR; resistance to two or more antimicrobials) was also observed among the isolates. The majority of enterococcal isolates were resistant to two antimicrobials, but MDR up to seven antimicrobials was also observed. MDR in *E. faecium* from dairy cattle faecal samples has been previously reported (Edrington *et al.* 2009). In that study, although very few isolates were MDR, one *E. faecium* isolate was resistant to up to nine different antimicrobials while one *E. durans* exhibited resistance to four antimicrobials. In the present study, the most common MDR pattern was FlaLin and other common MDR patterns contained some combination of the three antimicrobials (Fla, Lin, Tet) for which most of the resistance was observed. A few MDR patterns were composed of daptomycin or other antimicrobials used to treat gram-positive infections in humans. But none of the patterns contained the combination of a β-lactam antimicrobial coupled with an aminoglycoside (gentamicin) or a glycopeptide (vancomycin) which are two of the usual treatments for enterococcal infections in humans (Wilson *et al.* 1995).

Food animals have been implicated as a source of antimicrobial-resistant bacteria, and to fully understand the role that food animals have in the dissemination and perpetuation of antimicrobial resistance, bacteria from on-farm sources must continue to be studied. One goal of the NAHMS Dairy 2007 study was to estimate the prevalence of food-borne pathogens in faeces from dairy cattle. Although enterococci are considered commensal bacteria, they can cause infections in humans and animals. The results of this study show that there is a high prevalence of antimicrobial-resistant enterococci in the faecal material of dairy cattle. These enterococci could have a role in environmental contamination leading to mastitis in dairy cattle and also could be transmitted to humans via raw milk products or other forms of contamination (Tenhagen *et al.* 2006). The resistance genes contained within the antimicrobial-resistant enterococci could also be transferred to other bacteria including those that are implicated in human diseases. The extent of antimicrobial resistance in enterococci from food animals should be monitored to fully assess the role these animals have as reservoirs of resistant bacteria and their potential impact on humans.

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